A thermo-reversible hydrogel* against chronic, burn and combat wound associated pathogens, and its application to colonized wounds

Jeyachchandran Visvalingam, PhD[1]; Suresh Regmi, PhD[1]; Sheung Pui Lee, MSc[1]; Miloslav Sailer, PhD[1]; Marc Edwards, MBA; Raymond Abdo, DPM[2]; Greg Schultz, PhD[1]

[1]Kane Biotech Inc, Winnipeg, Manitoba, Canada
jvisvalingam@kanebiotech.com
[2]Mercy Hospital South, St Louis, MO, USA

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Abstract

The objective of this study was to evaluate thermo-reversible properties, antimicrobial and antibiofilm properties of an antimicrobial hydrogel, and its effect on colonized wounds. Thermo-reversible property of the hydrogel was assessed using Brookfield viscometer during temperature increment and decrement. *In vitro* antimicrobial property against major chronic, burn and combat wound pathogens was assessed using modified ASTM E235-03 method. Mature biofilm of these organisms grown on nitrocellulose membrane at 37°C for 72 h were treated with the thermo-reversible hydrogel (TRH) for 7 days and viable numbers were determined. A two-patient case study was conducted to assess effect of TRH application on bacterial colonization of foot and toe ulcers. Viscosity of TRH increased with temperature increment, becoming solid gel at 35°C. TRH reduced viable numbers of tested organisms by ≥5 log CFU in ≤30 min. Treatment with TRH reduced viable numbers of biofilms of all organisms by ≥4 log CFU at day 3 and no biofilm re-growth of these organisms was noted until day 7. In both case studies, fluorescent imaging of foot and toe ulcers showed bacterial colonization. No bacterial colonization was detected by fluorescent imaging after a week of TRH use. This study provides a direct correlation of *in vitro* antimicrobial and antibiofilm effects of TRH with reduction of bacterial colonization on wounds.

In Memory of Dr. Gregory Schultz, PhD
Chief Scientific Officer, Kane Biotech

It is with profound sadness we acknowledge the unexpected passing of Greg Schultz, a world-renowned expert on wound care and biofilms. Greg helped lead the way at Kane in advancing the development and commercialization of our coactiv+™ and DispersinB® technologies as part of his on-going search for the solution to the biofilm problem in healthcare. His contributions were significant and will be realized for decades to come. His loss will simply be immeasurable.

Introduction

Chronic, burn and combat wounds are often colonized by biofilm forming bacteria that can trigger the inflammatory process, leading to delayed wound healing. Infection is one of the leading causes of death in burn and combat wound patients[1,4]. Thus, providing an effective treatment option that provides moist wound environment along with preventing microbial colonization and reducing biofilm bioburden are considered key for improving wound healing outcomes. The objective of this study was to evaluate thermo-reversible properties, antimicrobial and antibiofilm properties of an antimicrobial thermo-reversible hydrogel (TRH), and its effect on colonized wounds.

Materials and Methods

Assessing Thermo-Reversible Property: Changes in viscosity of TRH was measured using Brookfield viscometer during temperature increment and decrement to assess thermo-reversible property.

Antimicrobial Activity Testing: Antimicrobial activity of TRH against major chronic, burn and combat wound pathogens, *Staphylococcus aureus*, Methicillin resistant *S. aureus* (MRSA), *Pseudomonas aeruginosa, Acinetobacter baumannii, and Klebsiella pneumoniae* was tested using modified ASTM E235-03 method.

Prolonged Antibiofilm Activity: Mature (72h) biofilm of *S. aureus*, MRSA, *P. aeruginosa, A. baumannii, and K. pneumoniae* were grown on nitrocellulose membrane[2] for 72h at 37°C. Each biofilm was treated with TRH for 7 days at 37°C and viable numbers were determined at day 0 before treatment, day 1, 3 and 7 after treatment. Viable numbers of untreated control biofilms of these organisms were also determined at the same sampling interval.

Case Studies: A two-patient clinical case study was conducted to determine how healing and bacterial colonization changed over the course of TRH application. Microbial colonization before and after TRH use was determined using a MolecuLight fluorescence imaging device[3]. The region of the wound with bacterial colonization can yield red or cyan fluorescence, while matrix components such as collagen, skin, fibrin, and slough yield green fluorescence.

Results

Thermo-Reversible Property: Gel was liquid below 18°C. Viscosity of gel progressively increased with temperature increment, reaching maximum of 17500 cP at 35°C. This property can help gel to remain on the wound. As temperature decreased, viscosity of the gel returned to a liquid state (Figure 1).

Antimicrobial Activity: TRH was effective against several chronic wound, combat trauma, and burn associated pathogens, showing >99.999% (5-$\log_{10}$) reduction in planktonic microbial numbers within 30 min (Table 1).

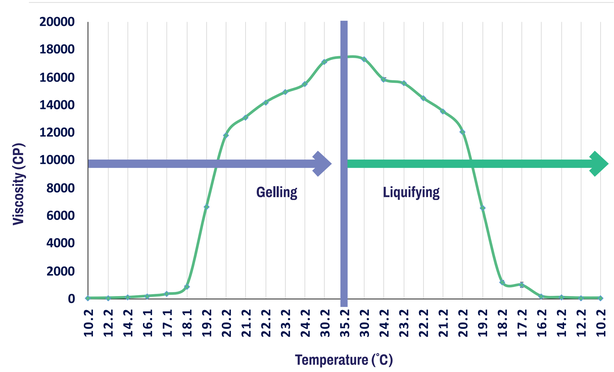

Figure 1. Changes in viscosity during temperature increment and decrement showing thermo-reversible property of TRH.

Table 1: Log CFU reductions observed after treating with TRH for 30 min

Organism	Log reduction (Log CFU/mL)
S. aureus	>5
MRSA	>5
P. aeruginosa	>5
A. baumannii	>5
K. pneumoniae	>5

Prolonged Antibiofilm Activity: Viable numbers of *S. aureus* reached below detection by day 3 and no regrowth was observed at day 7. Viable numbers of MRSA, *K. pneumoniae* and *A. baumannii* decreased by ≥7.9 $\log_{10}$ CFU by day 3 and biofilms of these organisms did not regrow until day 7. Viable numbers of *P. aeruginosa* decreased by ≥4 $\log_{10}$ CFU by day 1 and remained at that level for 7 days, indicating TRH prevented re-growth of *P. aeruginosa* biofilm (Fig 2)

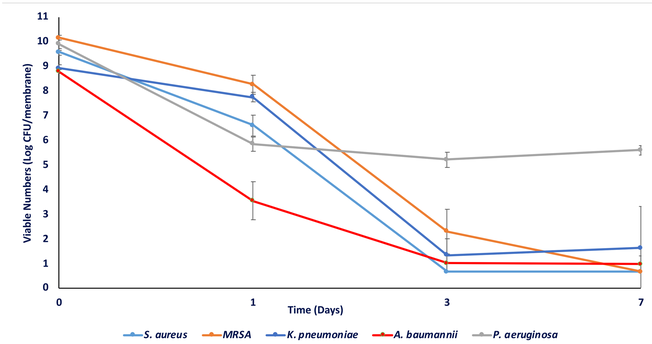

Figure 2. Effect of TRH against biofilms during prolonged exposure. Mature biofilm (72h) of wound related pathogens were grown on nitrocellulose membrane and treated with wound gel for 7 days.

Case Studies: In both case studies, fluorescent imaging of foot and toe ulcers showed bacterial colonization. No bacterial colonization was detected by fluorescent imaging after a week of TRH use (Figure 3).

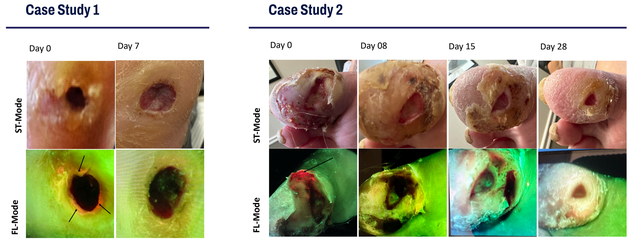

Figure 3. Changes in bacterial colonization and progress in wound (ulcer) healing before and after use of Antimicrobial hydrogel.

Conclusions

At 35 ˚C, TRH was thick and non-runny gel. This property can help gel to remain on wound. As it was shown in temperature decrement, viscosity of the gel decreased, which would aid removing gel from wound at dressing change with cold water or saline without harming wound tissue. Activity against biofilms was sustaining and lasted for up to 7 days. Case studies provided real-life evidence that antimicrobial hydrogel can remove bacterial colonization from foot and toe ulcers and can facilitate wound healing/closure. This study provides a direct correlation of *in vitro* antimicrobial and antibiofilm effects of TRH with reduction of bacterial colonization on wounds.

References

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